This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 215

See attached form for additional information.

Interagency Report Control No.: 12/28/05 JMK

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANNUAL REPORT OF RESEARCH FACILITY

(TYPE OR PRINT)

1. CERTIFICATE NUMBER: 74-R-0011

CUSTOMER NUMBER: 1382

FORM APPROVED
OMB NO. 0579-0036

b6,b7c

Alcon Research, Ltd
6201 South Freeway, R9-1
Fort Worth, TX 76134

12/8/05 JMK

Telephone: (817) -293-0450

COPY

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, or experimentation, or held for these purposes. Attach additional sheets if necessary)

FACILITY LOCATIONS (Sites) - See Atached Listing

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY **(Attach additional sheets if necessary or use APHIS Form 7023A)**

A. Animals Covered By The Animal Welfare Regulations	B. Number of animal being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not ye used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use o pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals an for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for wh the use of appropriate anesthetic, analgesic, or tranquiliz drugs would have adversely affected the procedures, res or interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress in these animals and the reasc such drugs were not used must be attached to this report	F. TOTAL NUMBER OF ANIMALS (COLUMNS C + D + E)
4. Dogs					
5. Cats			16		16
6. Guinea Pigs	130	65	1,978	68	2,111
7. Hamsters					
8. Rabbits	230	1,212	5,227		6,439
9. Non-human Primates	56		295		295
10. Sheep					
11. Pigs					
12. Other Farm Animals					
13. Other Animals					
Chinchillas			106		106

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anestetic, analgesic, and tranquilizing drugs, prior to, during, and following actual rese teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and app Institutional Animal Care and Use Committee (IACUC). **A summary of all such exceptions is attached to this annual report.** In addition to identifying the IACUC-approved exceptions, this summary in brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional Official)

(B)(6) (B)(7)(c)

DATE SIGNED 10-25-05

APHIS FORM 7023 (Replaces VS FORM 18-23 (OCT 88), which is obsolete.)
(AUG 91)

OCT 26 2005

Column E Explanation

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

1. **Registration Number:** 74-R-0011

2. **Number** 8 **of animals used in this study.**

3. **Species (common name)** Guinea Pig **of animals used in the study.**

4. **Explain the procedure producing pain and/or distress.**

   Guinea pigs are injected with approximately (b)(4) or horse serum (positive control) on three occasions into the peritoneal cavity. Subsequently, each test subject is injected intravenously and examined for anaphylactic response. Guinea pigs are humanely euthanized after the last regimen is completed. The eight animals in Column E are positive controls and are expected to exhibit symptoms of respiratory distress, collapse, and death. This procedure screens for potential ocular inflammation in raw material/finished product prior to market release for human use. This is mandatory by the Japanese Pharmacopoeia for product release in Japan.

5. **Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below).**

   The guinea pig is an established model for antigen-induced respiratory anaphylaxis. Anaphylaxis is the required outcome of the positive control for this study. No anesthetic, analgesic, or tranquilizing drugs are administered since these drugs potentially confound interpretation and conclusions derived from this testing. This test is employed in response to required safety testing by the Japanese Ministry of Health and Welfare for marketing viscoelastic products in Japan.

   The Guinea Pig Antigenicity Test, as described in the Japanese Pharmacopoeia (JP) XIV, states that the positive control animals exhibit symptoms of respiratory distress or collapse and not less than three animals are killed. The observation of signs of respiratory distress, collapse or death of the animals for 30 minutes after each intravenous injection and 24 hours later constitutes data points. The positive control is the only group that has exhibited distress and/or pain in the past five years. This group (8 guinea pigs) dies or is euthanized within a 30 minute window. The duration of distress and/or death is a maximum of 30 minutes. The JP specifies a 30 minute observation period. Use of anesthetic or tranquilizing drugs would interfere with the observations necessary to meet the JP criteria (respiratory distress and collapse.) Analgesics are not utilized due to the short duration of respiratory distress. Once respiratory distress is observed, collapse and death occur

rapidly. Historically, positive control animals expire within 10 minutes. Due to the short time between signs of respiratory distress and expiration, earlier endpoints are not feasible. As respiratory distress is the key observation in this positive control group, any interaction prior to respiratory distress would confound interpretation of the test. The number of tests is kept to a minimum and is conducted 1-3 times per year.

6. **What, if any, federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):**

The Guinea Pig Antigenicity Test has been registered as a specification and test method for finished product. Therefore, the test needs to be performed on the raw material that is used to manufacture finished product for Japan. PMA is the Pre Market Approval registry number assigned by the FDA (P840064, P890047 and P990023.)

Agency Japanese Ministry of Health and Welfare

CFR The Japanese Pharmacopoeia, 14th edition, 2001, page 399

THE JAPANESE PHARMACOPOEIA

第十四改正 日本薬局方

Official from April 1.2001

English Version

CONTENTS

Notice;This English Version of the Japanese Pharmacopoeia, Fourteenth Edition is published to meet the needs of the non-Japanese speaking people. When and if any discrepancy arises between the Japanese original and its English translation,the former is authentic.

The Japanese Pharmacopoeia documents are in Portable Document Format (PDF). To view or print these documents, you must use the Adobe Acrobat Reader.

Last updated:19,December 2001,
mail to jp14e@nihs.go.jp

methanol by warming, add 1 mL of Fehling's TS, and heat: a red precipitate is produced.

(3) Proceed with 0.01 g of Dexamethasone as directed under the Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the solution obtained responds to the Qualitative Tests for fluoride.

(4) Dissolve 1.0 mg of Dexamethasone in 10 mL of ethanol (95). Mix 2.0 mL of the solution with 10 mL of phenylhydrazine hydrochloride TS, heat in a water bath at 60°C for 20 minutes, and cool the solution. Determine the absorption spectrum of the solution as directed under the Ultraviolet-visible Spectrophotometry, using as the blank the solution prepared with 2.0 mL of ethanol (95) in the same manner as the former solution, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Dexamethasone Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) Determine the infrared absorption spectrum of Dexamethasone, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Dexamethasone Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Dexamethasone and Dexamethasone Reference Standard in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.

Optical rotation $[\alpha]_D^{20}$: +72 – +80° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

Purity (1) Heavy metals—Proceed with 1.0 g of Dexamethasone according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Other steroids—Dissolve 0.10 g of Dexamethasone in 10 mL of acetone, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane and methanol (45:4) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying Not more than 0.5% (0.2 g, 105°C, 3 hours).

Residue on ignition Not more than 0.1% (0.2 g, platinum crucible).

Assay Dissolve about 0.01 g each of Dexamethasone and Dexamethasone Reference Standard, previously dried and accurately weighed, in 70 mL each of diluted methanol (1 in 2), add exactly 5 mL each of the internal standard solution, then add diluted methanol (1 in 2) to make 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of these solutions as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dexamethasone to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{22}H_{29}FO_5 = \text{amount (mg) of Dexamethasone Reference Standard} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of propyl parahydroxybenzoate in diluted methanol (1 in 2) (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (2:1).

Flow rate: Adjust the flow rate so that the retention time of dexamethasone is about 6 minutes.

Selection of column: Dissolve 2 mg of methyl parahydroxybenzoate and 4 mg of ethyl parahydroxybenzoate in 100 mL of diluted methanol (1 in 2). Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of methyl parahydroxybenzoate and ethyl parahydroxybenzoate in this order with the resolution between these peaks being not less than 5.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Dextran 40

デキストラン 40

Dextran 40 is a product obtained by partial decomposition of polysaccharide, which is produced by fermentation of sucrose with *Leuconostoc mesenteroides* van Tieghem (*Lactobacillaceae*), and the average molecular mass is about 40,000.

When dried, it contains not less than 98.0% and not more than 102.0% of dextran 40.

Description Dextran 40 occurs as a white, amorphous powder. It is odorless and tasteless.

It is practically insoluble in ethanol (95) and in diethyl ether.

It dissolves gradually in water.

It is hygroscopic.

Identification To 1 mL of a solution of Dextran 40 (1 in 3000) add 2 mL of anthrone TS: a blue-green color develops and turns gradually dark blue-green. Then to this solution add 1 mL of diluted sulfuric acid (1 in 2) or 1 mL of acetic acid (100): the solution does not change in color.

pH Dissolve 1.0 g of Dextran 40 in 10 mL of water: the pH of this solution is between 5.0 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Dextran 40 in 10 mL of water by warming: the solution is clear and colorless.

(2) Chloride—Perform the test with 2.0 g of Dextran 40. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid (not more than 0.018%).

(3) Heavy metals—Proceed with 1.0 g of Dextran 40 according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic—Prepare the test solution with 1.5 g of Dextran 40 according to Method 1, and perform the test using Apparatus B (not more than 1.3 ppm).

(5) Nitrogen—Weigh accurately about 2 g of Dextran 40, previously dried, and perform the test as directed under the Nitrogen Determination, where 10 mL of sulfuric acid is used for decomposition, and 45 mL of a solution of sodium hydroxide (2 in 5) is added: the amount of nitrogen (N: 14.01) is not more than 0.010%.

(6) Reducing substances—Weigh exactly 3.00 g of Dextran 40, previously dried, dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh exactly 0.450 g of glucose, previously dried, dissolve in water to make exactly 500 mL, and use this solution as the control solution. Pipet 5 mL each of the sample solution and the control solution, and add water to make exactly 50 mL, respectively. Pipet 5 mL each of these solutions, add 5 mL of alkaline copper TS, exactly measured, and heat for 15 minutes in a water bath. After cooling, add 1 mL of a solution of potassium iodine (1 in 40) and 1.5 mL of dilute sulfuric acid, and titrate with 0.005 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS).

The titrant consumed for the sample solution is not less than that for the control solution.

Loss on drying Not more than 5.0% (1 g, 105°C, 6 hours).

Residue on ignition Not more than 0.10% (1 g).

Viscosity (1) Dextran 40—Weigh accurately 0.2 to 0.5 g of Dextran 40, previously dried, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Perform the test with the sample solution and with water as directed in Method 1 under the Viscosity Determination at 25°C : the intrinsic viscosity is between 0.16 and 0.19.

(2) High-molecular fraction—Weigh accurately about 6 g of Dextran 40, previously dried, dissolve in water to make exactly 100 mL, and transfer to a flask. Add slowly enough methanol to get 7% to 10% of the precipitate (usually 80 to 90 mL) at 25 ± 1°C with stirring. Dissolve the precipitate at 35°C in a water bath with occasional shaking, and allow to stand for more than 15 hours at 25 ± 1°C. Remove the supernatant liquid by decantation, and heat the precipitate of the lower layer to dryness on a water bath. Dry the residue, and determine the intrinsic viscosity of the dried substance as directed in (1): the value is not more than 0.27.

(3) Low-molecular fraction—Weigh accurately about 6 g of Dextran 40, previously dried, dissolve in water to make exactly 100 mL, and transfer to a flask. Add slowly enough methanol to get 90% to 93% of the precipitate (usually 115 to 135 mL) at 25 ± 1°C with stirring, centrifuge at 25°C, and evaporate the supernatant liquid to dryness on a water bath. Dry the residue, and determined the intrinsic viscosity of the dried substance as directed in (1): the value is not less than 0.09.

Antigenicity Dissolve 10.0 g of Dextran 40 in isotonic sodium chloride solution to make 100 mL, sterilize, and use this solution as the sample solution. Inject 1.0 mL of the sample solution on 3 occasions at intervals of 2 days into the peritoneal cavity of each of 4 well-nourished, healthy guinea pigs weighing 250 to 300 g. Inject 0.10 mL of horse serum into the peritoneal cavity of each of 4 guinea pigs of another group as a control. Inject 0.20 mL of the sample solution intravenously to each of 2 guinea pigs of the first group 14 days after the first intraperitoneal injection and into each of the remaining 2 guinea pigs 21 days after the injection, and inject 0.20 mL of horse serum intravenously in the same manner into each guinea pig of the second group. Observe the signs of respiratory distress, collapse or death of the animals for 30 minutes after each intravenous injection and 24 hours later: the animals of the first group exhibit no signs mentioned above.

All the animals of the second group exhibit symptoms of respiratory distress or collapse and not less than 3 animals are killed.

Pyrogen Dissolve 10.0 g of Dextran 40 in isotonic sodium chloride solution to make 100 mL, and perform the test: this solution meets the requirements of the Pyrogen Test.

Assay Weigh accurately about 3 g of Dextran 40, previously dried, dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Determine the optical rotation α_D with the sample solution as directed under the Optical Rotation Determination in a 100-mL cell at 20 ± 1°C.

$$\text{Amount (mg) of dextran 40} = \alpha_D \times 253.8$$

Containers and storage Containers—Tight containers.

Dextran 40 Injection

デキストラン 40 注射液

Dextran 40 Injection is an aqueous solution for injection. It contains not less than 9.5 w/v% and not more than 10.5 w/v% of dextran 40.

Method of preparation

Dextran 40	10 g
Isotonic Sodium Chloride Solution	a sufficient quantity
	To make 100 mL

Prepare as directed under Injections, with the above ingredients.

No preservative is added.

Description Dextran 40 Injection is a clear and colorless liquid. It is slightly viscous.

Identification (1) Dilute 1 mL of Dextran 40 Injection with water to 200 mL, and to 1 mL of the diluted solution add 2 mL of anthrone TS: a blue-green color develops and turns gradually dark blue-green. Add 1 mL of diluted sulfuric acid (1 in 2) or 1 mL of acetic acid (100) to this solution: the solution does not change in color.

(2) Dextran 40 Injection responds to the Qualitative

Column E Explanation

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

1. **Registration Number:** 74-R-0011

2. **Number** 60 **of animals used in this study.**

3. **Species (common name)** Guinea Pig **of animals used in the study.**

4. **Explain the procedure producing pain and/or distress.**

   Guinea pigs are actively sensitized by a single intraperitoneal injection of ovalbumin antigen-$ALOH_3$ adjuvant mixture. A minimum of two weeks later one eye only is topically challenged with ovalbumin and the resulting allergic response is quantified. The guinea pigs are humanely euthanized at the completion of the study. The 60 animals in Column E are positive controls and are expected to exhibit clinical signs of allergic response, congestion, edema and discharge.

5. **Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below).**

   Sensitized guinea pigs have been shown to have an enhanced vascular permeability response upon antigen challenge. Edema, induced by cellular influx, is one of the required outcomes of the positive control of this model. Analgesic compounds are not administered since they are known to possess anti-inflammatory activity which would hinder local mediator release and/or action thereby reducing cellular influx.

   This is a drug screening model to evaluate unknown molecules in an in vivo model of allergic conjunctivitis in which the drugs are administered topical ocular. Vehicle groups in which mast cells degranulate and functioning nerve ending cause a release of neural peptides are necessary to interpret the results. Topical ophthalmic anesthetics cannot be administered as they would act directly on the nerve endings, blocking the release of neural peptides. Treating only the vehicle group with an opiate-based analgesic or an analgesic without anti-inflammatory effect, such as oral acetaminophen, would add an additional variable to the experiment. Therefore, all the groups would have to be treated the same. This leads to the possibility of a drug interaction with the opiate or acetaminophen as they are known to have interactive effects with other drugs. This could lead to an incorrect analysis of the data.

6. **What, if any, federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):**

Agency N/A

CFR N/A